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THE EFFECT OF MEAT AND OF MEAT EXTRACT MEDIA UPON THE FERMENTATIVE ACTIVITY OF STREPTOCOCCI.*

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During the past few years several papers have appeared dealing with the fermentative reactions of streptococci in special media. The results obtained by different workers have proven discouragingly difficult to align on any common basis, such as origin. My own work now includes about 700 strains of streptococci from various sources, chiefly milk, blood, and the mucous membranes and the feces of animals, man included. Concerned, therefore, about this lack of correspondence, I sent a few throat strains to Professor C. N. Hilliard of Purdue University in order to check my own procedure. The strains were selected from throat strains on hand, and with reference to their raffinose-fermenting power, as our previous work showed a decided difference in the percentage of raffinose fermenters.

Duplicate subcultures of the strains chosen were incubated for 24 hours. One of each pair was sent to Professor Hilliard. Three days later we transferred our cultures to fresh agar slants (made with meat, not meat extract). Twenty-four hours later these fresh agar cultures were used to inoculate the Gordon media (sugar-free broth containing 1 per cent saccharose, lactose, salicin, raffinose, or mannite). The results (Table 1) proved very puzzling until Professor Hilliard[1] wrote that his Gordon media had been made from meat *extract*. For my Gordon media *meat* was used.

It will be noticed that all the substances fermented in the Gordon meat media show a decided falling-off in the Gordon meat extract

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[1] Professor Hilliard's records for meat extract are the averages of two duplicate Gordon tubes. My meat records are of single Gordon tubes, except 98 and 106, each of which gives the averages for two strains done in duplicate at the time of isolation and continued for six weeks as separate cultures; their titration records were practically alike. All cultures were incubated 3 days at 37°, and titrated in the cold, against n/20 phenolphthalein. The initial acidity has been subtracted from all results given in the tables.

media, as in Strain 81 where the raffinose titration record falls from 5.2 to 1.7, or in Strain 99 where the saccharose record of 2.8 falls to 0.7. Substances not at all or barely fermented in the meat media (−0.1 to 0.5) are not noticeably affected by the meat extract, though raffinose and inulin sometimes show a slight increase. (A slight increase is also seen in mannite for 106; earlier [January] records for 106 in mannite were 0.0.)

TABLE 1.

TITRATION RECORDS OF SEVEN THROAT STRAINS OF STREPTOCOCCI, FEBRUARY, 1913.

Strain	Source	Date Isolated	Medium	Saccharose	Lactose	Salicin	Raffinose	Mannite
20........	Throat, man; suspected diphtheria	Oct., 1912	Meat	3.5	3.8	4.0	2.5	2.7
			Meat extract	1.0	1.3	1.3	0.9	1.2
67........	Palate, Cat 4	Jan., 1913	Meat	4.0	4.2	4.1	0.6	3.6
			Meat extract	1.3	1.8	1.3	0.0	1.6
81........	Tonsils, Cat 4	" "	Meat	5.0	5.2	4.2	5.2	0.1
			Meat extract	2.3	2.3	1.8	1.7	0.2
98........	Palate, Cat 6	" "	Meat	3.3	2.5	2.1	2.4	2.1
			Meat extract	0.8	0.8	0.7	0.7	0.8
99........	Palate, Cat 6	" "	Meat	2.8	2.5	1.9	2.4	2.1
			Meat extract	0.7	0.9	0.7	0.7	0.8
100........	Throat, Cat 1	" "	Meat	4.1	4.3	4.4	0.2	3.5
			Meat extract	1.0	1.8	1.9	0.0	1.5
106........	Tonsils, Cat 6	" "	Meat	4.5	3.4	4.3	3.9	0.8
			Meat extract	1.6	1.8	1.4	2.3	1.4

For these different results the only explanation seemed to be the respective uses of meat and of meat extract. The initial acidity varied about 0.2, an insignificant matter if the fermentative activity can increase it to 5.2 as in 81, lactose (Table 1); Professor Hilliard's media had the *lower* and presumably more favorable initial acidity (0.3–0.5).

Later, to try out more fully these differences apparently caused by meat and by meat extract media, I selected from my stock cultures a series in which the strains differed in their original fermentative results, ranging from a non-fermenter (Strain 98, Table 2) to one that fermented six of the Gordon test media (Strain 71, Table 2). They varied also in the time under cultivation and in origin as described in the table. To subject the strains for a slightly longer period, contemporary subcultures were first made in plain broth, one lot from meat and the other from meat extract.

After 24 hours, corresponding meat and meat extract Gordon media were inoculated from the two sets of broth tubes. The conditions[1] were the same in every other respect. The titration results (Table 2) at the end of the usual time (3 days) showed practically the same differences as in the earlier series.

TABLE 2.

TITRATION RECORDS OF STREPTOCOCCI FROM VARIOUS SOURCES, SEPTEMBER, 1913.

Strain	Source	Date Isolated	Medium	Saccharose	Lactose	Salicin	Raffinose	Mannite	Inulin
11......	Milk	Oct., 1912	Meat	0.0	0.4	3.4	0.1	0.1	0.1
			Meat extract	0.1	0.5	1.7	0.3	0.2	0.2
......	Intestine, dog	March, 1913	Meat	−0.1	3.6	3.0	−0.1	0.0	0.1
			Meat extract	0.2	1.6	1.8	0.3	0.1	0.2
9......	Throat, man	“ “	Meat	4.4	0.2	3.0	0.1	0.0	0.0
			Meat extract	1.8	0.1	1.6	1.4	0.0	0.0
42......	Mastoid abscess, man	March, 1911	Meat	2.8	2.8	−0.1	2.5	0.1	0.3
			Meat extract	1.3	1.1	0.0	1.0	0.1	0.2
61......	Milk	May, 1913	Meat	−0.1	3.3	0.0	0.2	0.3	0.0
			Meat extract	−0.1	1.2	0.1	0.1	0.2	0.2
71......	Feces, equine	“ “	Meat	3.3	3.2	3.8	2.5	2.9	2.3
			Meat extract	1.4	1.5	1.5	1.1	1.1	0.9
88......	Feces, human	“ “	Meat	0.0	3.1	3.8	0.1	2.9	0.0
			Meat extract	1.0	1.7	2.2	0.8	1.2	1.2
98......	Throat, dog	“ “	Meat	−0.1	0.0	0.0	0.0	0.0	0.0
			Meat extract	0.0	0.0	−0.1	−0.1	−0.1	0.0
103......	Milk	June, 1913	Meat	3.4	3.3	2.4	0.0*	0.0	0.0
			Meat extract	1.4	1.4	1.0	1.0	0.2	0.5
111......	Anthrax blood	“ “	Meat	0.6*	2.7	3.1	0.2	2.3	0.1
			Meat extract	0.8	0.9	1.3	0.8	0.8	0.8
233......	Duodenum, dog	August, 1913	Meat	2.9	2.8	2.9	0.1	0.1	−0.1
			Meat extract	1.2	1.0	1.0	0.6	0.3	0.6

* Previously fermented; titration record of 103 raffinose = 3.4; of 111 saccharose = 1.8.

Smears were made of all my Gordon media before titration. In this preliminary report it is sufficient to state that later examination testified to the purity[2] of the cultures, and indicated that physiological differences might be expected. Slight but not always parallel differences in the gross appearances of both the plain broth and the Gordon media were noted. These, too, do not materially affect the significance of the titration results.

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[1] The same stock of substances (peptone, inulin, etc.) was used throughout. The only difference, as before stated, was that meat (15 pounds to 13 liters) was used in one case, and meat extract (3 gm. to 1 liter) in the second case. The sugar-free media (*B. coli*, 7 days at 37°) were filtered through a Berkefeld filter. All the media used were sterilized in the Arnold, not in the autoclav, and for like periods of time.

[2] Before inoculation, to insure sterility, all of the Gordon tubes were incubated at 37° for one day; they had been sterilized but 30 minutes on two consecutive days.

The depression of the fermentative activities is evidently not permanent. A third short series (not given here), in which the organisms were kept for six days (two transfers) in meat and in meat extract broth and then incubated in Gordon media made of meat, showed no final titration differences.

Publication of data based on so few strains is justified, I feel, in this case by two facts: First, the differences are not only most marked (often reaching 200 to 300 per cent), but they are remarkably uniform in amount and direction. Second, other and longer series to test constancy and the influence of age, temperature, and the kind of medium show no such marked or uniform effect.

In the last few years a number of bacteriologists have been interested in the fermentative powers of the streptococci; several papers on this subject have appeared, and other papers, read during the last year in New York and Washington, have not yet found their way into print. The difficulty one finds in lining up his work with that of the other workers is due, I suspect, to the fact that some have used meat, others meat extract, in making up their media. Litmus and phenolphthalein, too, further complicate the end results. With meat media it would make less difference which indicator was used. But in meat extract media, where the final acidity record often drops down to 0.7–1.0, litmus with its higher neutral point gives no indication of the possible powers of the strain under investigation.

CONCLUSIONS.

The fermentative activities of streptococci vary greatly with the use of meat or of meat extract in making the special media. All workers should, therefore, state definitely whether meat or meat extract media were used throughout such investigations.

Qualitative results, estimated with litmus as an indicator, are not comparable with quantitative ones, with phenolphthalein as an indicator, if meat extract is used in making the special media.